

Appln No. 10/817,297
Amdt date November 9, 2005
Reply to Office action of June 9, 2005

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Please amend claims 14, 18, 21, 22, 24 and 26.

1. (Original) A method of monitoring cellular activity in a cellular specimen, comprising:

applying a plurality of different excitable markers to the specimen;

focusing light upon a region of the specimen from a laser microscope to excite the markers in the region and cause fluorescence to be radiated by the markers in the region;

separating the fluorescence into wavelength bands using a tunable filter;

detecting the fluorescence through an array of detectors, with each detector receiving one of the wavelength bands and generating a corresponding signal; and

analyzing the detected fluorescence to qualitatively and quantitatively identify the contribution to the fluorescence from each of the plurality of different excitable markers.

2. (Original) The method of claim 1, wherein the step of analyzing includes quantitatively determining an intensity contribution to the fluorescence from each of the plurality of different excitable markers.

Appln No. 10/817,297

Amdt date November 9, 2005

Reply to Office action of June 9, 2005

3. (Original) The method of claim 2, wherein the step of analyzing uses a linear unmixing operation.

4. (Original) The method of claim 3, wherein the linear unmixing operation includes the steps of:

storing the detected fluorescence in a memory;

comparing the stored fluorescence with a model fluorescence generated from a plurality of templates characteristic of a fluorescence spectrum of each of the plurality of different excitable markers;

varying the weights of each of the plurality of templates until the model fluorescence closely matches the stored fluorescence; and

determining quantitative information about the intensity contribution to the fluorescence from each of the plurality of different excitable markers based on the weights.

5. (Original) The method of claim 1, wherein the step of analyzing uses a principal component analysis of the fluorescence.

6. (Original) The method of claim 1, wherein separating the fluorescence includes using one or more of either a grating or prism.

7. (Original) The method of claim 1, wherein separating the fluorescence includes using a dichromatic mirror.

Appln No. 10/817,297

Amdt date November 9, 2005

Reply to Office action of June 9, 2005

8. (Original) The method of claim 1, wherein the tunable filter is a liquid crystal filter.

9. (Original) The method of claim 1, wherein the tunable filter is an acousto-optical filter.

10. (Original) The method of claim 1, wherein applying a plurality of excitable markers includes applying a plurality of fluorescent probes to the specimen.

11. (Original) The method of claim 1, wherein detecting the fluorescence includes using a plurality of photomultiplier tubes.

12. (Original) The method of claim 1, wherein detecting the fluorescence includes using a plurality of high gain photomultiplier tubes.

13. (Original) The method of claim 1, wherein focusing light to the specimen comprises focusing light from a two-photon laser microscope.

14. (Currently Amended) A system for monitoring cellular activity in a cellular specimen that contains a plurality of excitable markers, the system comprising:

Appln No. 10/817,297

Amdt date November 9, 2005

Reply to Office action of June 9, 2005

a laser microscope that is operative to excite the markers in a region of the specimen, wherein the markers in the region radiate fluorescence as a result;

a tunable filter that is operative to process the fluorescence and to pass a portion of the fluorescence, wherein the portion of the fluorescence is within a wavelength band that depends on the setting of the filter;

a plurality of detectors operative to receive the processed fluorescence and to convert the fluorescence into a corresponding plurality of signals; and

an analyzer that is operative to receive the plurality of signals ~~signal~~ and to qualitatively and quantitatively identify the contribution to the fluorescence from each of the plurality of different excitable markers.

15. (Original) The system of claim 14, wherein the analyzer is operative to quantitatively determine an intensity contribution to the fluorescence from each of the plurality of different excitable markers.

16. (Original) The system of claim 15, wherein the analyzer uses a linear unmixing operation.

17. (Original) The system of claim 16, wherein the analyzer comprises:

a memory operative to store the detected fluorescence;

a processor operative to generate a model fluorescence from a plurality of templates characteristic of a fluorescence

Appln No. 10/817,297

Amdt date November 9, 2005

Reply to Office action of June 9, 2005

spectrum of each of the plurality of different excitable markers, compare the model fluorescence with the stored fluorescence, vary the weights of each of the plurality of templates until the model fluorescence closely matches the stored fluorescence, and determine quantitative information about the intensity contribution to the fluorescence from each of the plurality of different excitable markers based on the weights.

18. (Currently Amended) The ~~method~~ system of claim 14, wherein the analyzer uses a principal component analysis.

19. (Original) The system of claim 14, wherein the tunable filter comprises a liquid crystal tunable filter.

20. (Original) The system of claim 14, wherein the tunable filter comprises an acousto-optical tunable filter.

21. (Currently Amended) The system of claim 14, wherein the plurality of detectors ~~comprise~~ comprises a plurality of photomultiplier tubes.

22. (Currently Amended) The system of claim 14, wherein the plurality of detectors ~~comprise~~ comprises a plurality of high-gain photomultiplier tubes.

23. (Original) The system of claim 14, wherein the laser microscope comprises a multi-photon laser microscope.

Appln No. 10/817,297

Amdt date November 9, 2005

Reply to Office action of June 9, 2005

24. (Currently Amended) The system of claim 14, ~~therein~~
~~the system~~ further ~~comprises~~ comprising a collector that at
least substantially envelops the specimen to receive
fluorescence from the markers.

25. (Original) The system of claim 24, wherein the
collector comprises an integrating sphere.

26. (Currently Amended) A laser scanning microscope for
monitoring cellular activity in a cellular specimen that
contains a plurality of excitable markers, the microscope
comprising:

a laser operative to excite the markers in a region of the
specimen, wherein the markers in the region radiate fluorescence
as a result;

a tunable filter that is operative to process the
fluorescence and to pass a portion of the fluorescence, wherein
the portion of the fluorescence is within a wavelength band that
depends on the setting of the filter;

a plurality of detectors operative to receive the processed
fluorescence and to convert the fluorescence into a
corresponding plurality of signals; and

an analyzer that is operative to receive the plurality of
signals ~~signal~~ and to qualitatively and quantitatively identify
the contribution to the fluorescence from each of the plurality
of different excitable markers.

Appln No. 10/817,297

Amdt date November 9, 2005

Reply to Office action of June 9, 2005

27. (Original) The microscope of claim 26, wherein the analyzer is operative to quantitatively determine an intensity contribution to the fluorescence from each of the plurality of different excitable markers.

28. (Original) The microscope of claim 26, wherein the analyzer uses a linear unmixing operation.